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## Use of FT-NIRS for determination of chemical components and nutritional value of natural pasture

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### ABSTRACT

This study evaluates the potential of Fourier transformation near-infrared reflectance spectroscopy to estimate the nutritional value and the chemical composition of natural pastures. Variability from all samples of pastures available is considered in order to assess the applicability of the calibration models in the future predictions. Chemical components (dry matter, crude protein, ash, ether extract, crude fibre, fibrous fractions) of grass samples were determined by applying official methods, and milk and meat forage units were calculated. Calibration and validation models were developed between chemical–nutritional parameters and NIRS spectral data using partial least square regression (PLS). The capacity of methods has been achieved using two validation approaches: the first using an independent dataset for prediction and the second by cross-validation process. The results are evaluated in term of coefficient of determination, root-mean-square error and residual prediction deviation. Despite the wide variability of the data set, the results of FT-NIRS have been able to estimate the chemical composition of natural and naturalised pasture with good accuracy and precision, while for nutritional value parameters, a further evaluation may be useful.

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### KEYWORDS

FT-NIRS; natural pasture; nutritional value; chemical components; quality evaluation

### Introduction

In Tuscany, marginal productive systems are traditionally based on grazing of natural pastures that represent a significant resource for many farms. Pasture nutritional quality varies as result of biotic and abiotic factors including herbage species and nutrients availability, stage of maturity, topography and climatic conditions.

Wet chemistry is traditionally used to characterise chemical composition of animal feed but these techniques are time-consuming, expensive, destructive and non-adapted for a real-time feed analysis. These limits are even more apparent when associated with natural pastures that change their composition continually.

Near-infrared spectroscopy (NIRS) has become widely recognised as a valuable method in the determination of the chemical composition of a wide range of forage. Today, the Fourier transformation NIRS (FT-NIRS) seems to enable both qualitative and quantitative analyses, including improvements in signal-to-noise ratio, spectral resolution, wavelength accuracy and a reduction of time scan. The complexity of this technique application is due to the need of multivariate

analysis method that converts NIR spectral absorption into laboratory information. The development of robust and accurate NIRS predictions depends upon a database of sample handling that simultaneously will represent all characteristics of predicted forage.

Several authors have tested NIRS on feedstuffs and some publications are available for complex botanical resources (Danieli et al. 2004; Andrés et al. 2005; Lobos et al. 2013). However, not many publications consider all sample variables (stage of maturity, diverse geographical proveniences, botanical complex) and, as far as we know, only Danieli et al. (2004) consider all the constituents of the proximate analysis. The aim of this research is to evaluate the potential of FT-NIRS for the prediction of the chemical composition and nutritional value (milk and meat forage units) of pastures that can be a powerful tool at practical level.

### Materials and methods

#### Forage sample sets

The study used 105 samples collected in the period 2013–2014 in natural and naturalised pastures of

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Tuscany (Italy). The samples are harvested in main hills and mountains area of Tuscany where pastures are dedicated to animal grazing or having the potential to be grazed. The territories investigated are located at an altitude between 300 and 1174 m.a.s.l. including 30 mountain pastures and 23 hill pastures situated in 7 out of 10 provinces of Tuscany. Almost all pastures were sampled two times including both primary growth and secondary regrowth. Samples obtained by an area of 1 m<sup>2</sup> were representative of pastures including different proportions of herbaceous species evaluated on growth stage of principal botanical family as reported by INRA (2010). The proportion of each species in collected samples was highly variable. Predominant herbage species were as follows: *Avena fatua* L, *Capsella bursa pastoris* L, *Dactylis glomerata* L, *Festuca ovina* L, *Festuca pratensis* H, *Holcus lanatus* L, *Lolium perenne* L, *Poa pratensis* L, *Poa annua* L, *Trifolium pratense* L, *Trifolium repens* L, *Ranunculus bulbosus* L, *Taraxacum officinale* GH Weber ex Wiggers.

### Sample preparation and analysis

Each sample was dried in a forced air oven at 60 °C to constant weight, then grounded through a mill (Brabender OHG, Duisburg, Germany) to pass 1mm and analysed for the main nutritional parameters. The chemical analysis was determined according to the CEE-ASPA protocol (Martillotti et al. 1987). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were measured using the procedure described by Van Soest (1991). Nutritional value, expressed as milk forage unit (UFL) and meat forage unit (UFV), was determined according to INRA (2010), using ADF value for organic matter digestibility estimation.

### FT-NIRS data acquisition

For each sample, three aliquots of 4–6g of dried and grounded sample were exposed to an electromagnetic scan in the absorbance mode using a FT-NIRS Antaris II model (Thermo Fisher Scientific). Samples were presented in a circular cup spinner with a quartz window. Each spectral measurement was obtained from 32 scans performed at a wave numbers resolution of 16 cm<sup>-1</sup> over the range of 4000–9999 cm<sup>-1</sup> and corrected against the background spectrum of room environment which was performed routinely. Statistical analysis was implemented using the average spectrum of the three different replications.

### Calibration

Calibration and validation models were obtained correlating NIRS preprocessed spectral data with results from the chemical analysis for DM, CP, ash, CF, EE, NDF, ADF, ADL and from UFL and UFV values. For each chemical constituent, an individual model was developed. Multiplicative scatter correction (MSC) to all spectrum was applied, in order to eliminate optical interference, and removes physical effects like particle size and surface blaze at spectra wavelengths that do not transmit chemical or other information. Moreover, a set of outliers' spectrum were identified and removed when necessary. Finally, mathematical spectra pretreatments, based on first order derivatives, were used in order to optimise the extraction of useful information from the spectra. The application of mathematical pretreatment to spectra and evaluation of outliers were performed using the chemometrics software TQ Analyst 8.6.12 (Thermo Fisher Scientific 2011).

The models were calibrated and validated using two approaches. The first partitioned the total number of samples in two randomly datasets: one for calibration assembled of 80% of samples and the other 20% used in validation test in order to evaluate the prediction performance. Second approach used all the samples as training set in calibration, followed by fully cross-validation of the model using the 'leave-one-out' method where a single sample is removed from the data set and the model rebuilt without the sample. For each chemical constituent, the optimum number of PLS factors used for model development was the one that determined the lowest error.

Partial least squares (PLS) regression was applied both in the calibration and in the two validation models using TQ Analyst 8.6.12 (Thermo Fisher Scientific 2011). The best model of each parameter was selected on the lower root-mean-square error and on the highest calibration coefficient, respectively, in calibration (RMSEC – R<sup>2</sup>c), in prediction (RMSEP – R<sup>2</sup>p) and in cross-validation (RMSECV – R<sup>2</sup>CV). Root-mean-square error evaluates the performance of model regression and it expresses the differences between the predicted value and the measured references values (Lobos et al. 2013). Goodness and accuracy of models were tested using the residual prediction deviation (RPD) calculated as the ratio of the standard deviation of reference values to the mean error in calibration (RMSEC) and in prediction (RMSEP).

### Results and discussion

Mean, standard deviation, range, variation coefficient of chemical data and nutritional value of samples are

shown in Table 1. The results of traditional chemical analyses show a wide range of values for all the parameters. This result is straightforward considering the high biodiversity found in natural pastures and the highly different botanical composition but also the response to tissue ageing is due to differences in maturity stage of samples. Summary statistics for calibration (CAm) and prediction (CPm) models are shown in Table 2. The CPm model uses a randomly selected set of data not included in the CAm. Calibration and CPm models, as well as RPD, showed high accuracy for chemical parameters, whereas accuracy of UFL and UFV resulted lower.

Summary statistics for cross-validation models (CVm), as well as optimal number of PLS factors obtained by process, are shown in Table 3. According to Lobos et al. (2013), PLS regression shows a good capacity to predict chemical constituent of herbage, whereas some authors (Danieli et al. 2004) suggest that multiple linear regression (MLR) models fit chemical data better than the other models.

Dry matter estimation is possible thanks to the strong -OH absorption of the constituent. The model obtains similar result both with CAm ( $R^2$ : 0.997; RMSE: 0.937) and CPm ( $R^2$ : 0.993; RMSE: 0.973). The coefficient of determination for CAm is 10–15% higher than those reported by Fekadu et al. (2010) and Lobos et al. (2013), whereas RMSE was similar or slightly higher. This is probably due to the narrow range of values considered even if our RPDs are always higher. In cross-validation models, coefficient of determination CPm (0.957) is slightly lower than previous models, while the RMSECV (0.980) is similar. This suggests that validation based on unknown independent samples obtains most accurate model of prediction than those using cross-validation, according to Lobos et al. (2013).

Coefficient of determination and RMSE of crude protein, linked to the N-H adsorption (Roberts et al. 2003), were 0.995 and 0.585, respectively. RMSE of CAm was lower to what reported by Fekadu et al. (2010), Andrés et al. (2005) and Danieli et al. (2004) who obtained 0.92, 1.02 and 4.91 working on pastures located in Ethiopia, Spain and Italy, respectively. RMSE does not significantly differ between CAm (0.585) and

CPm (0.592) models and these differences are always smaller than the limit of 20% proposed by Moya (1993). This result is also supported by the high values found for RPD (10.3; 8.6), suggesting an advanced accuracy of the models. Also CVm presented a high value of  $R^2$  (0.977), which is higher than the level proposed as satisfactory by Andrés et al. (2005) in his evaluation on pasture CP contents (i.e. 0.97).

Ash, compared to CP and DM, shows slightly lower coefficient of determination ( $R^2$ <sub>c</sub>: 0.978;  $R^2$ <sub>p</sub>: 0.965;  $R^2$ <sub>CV</sub>: 0.753) for all the models considered and this could be due to the absence of absorption in the near-infrared region for the minerals. However, it is still possible an estimation of ash contents probably

**Table 2.** Summary statistics for the PLS calibration and prediction models.

Parameter	FPLS	Calibration (CAm)			Prediction (CPm)		
		$R^2$ <sub>c</sub>	RMSE	RPD	$R^2$ <sub>p</sub>	RMSEP	RPD
DM	8	0.997	0.937	11.8	0.993	0.973	9.4
CP	8	0.995	0.585	10.3	0.992	0.592	8.6
Ash	8	0.978	0.301	5.1	0.965	0.333	3.7
EE	8	0.989	0.091	7.0	0.982	0.137	4.6
CF	5	0.979	1.200	5.2	0.960	1.470	3.9
ADF	7	0.991	1.120	7.4	0.988	1.140	6.8
NDF	6	0.994	1.150	9.1	0.990	1.330	7.0
ADL	8	0.991	0.271	8.5	0.983	0.500	5.3
UFL	6	0.823	0.106	1.6	0.825	0.101	1.7
UFV	7	0.823	0.119	1.5	0.812	0.112	1.6

CAm: calibration models; CPm: external validation (prediction models); FPLS: number of PLS factors;  $R^2$ <sub>c</sub>: coefficient of determination in calibration;  $R^2$ <sub>p</sub>: coefficient of determination of prediction (external validation); RMSE: root-mean-square error of calibration; RMSEP: root-mean-square error of prediction (external validation); RPD: residual prediction deviation.

**Table 3.** Summary statistics of cross-validation of the PLS calibration model (CVm).

Parameter	FPLS	$R^2$ <sub>CV</sub>	RMSECV
DM	8	0.957	0.980
CP	8	0.977	1.210
Ash	8	0.753	0.858
EE	8	0.924	0.245
CF	5	0.939	1.960
ADF	7	0.948	2.490
NDF	6	0.958	2.930
ADL	8	0.926	0.913
UFL	6	0.768	0.120
UFV	7	0.778	0.126

CVm: cross-validation models; FPLS: number of PLS factors;  $R^2$ <sub>CV</sub>: coefficient of determination in cross-validation; RMSECV: root-mean-square error of cross-validation.

**Table 1.** Descriptive statistics of samples' composition.

	DM	CP	Ash	EE	CF	ADF	NDF	ADL	UFL	UFV
No.	105	105	103	105	98	103	101	100	98	100
Mean <sup>a</sup>	27.06	13.89	10.01	1.91	27.58	34.49	51.99	5.72	0.87	0.76
SD <sup>a</sup>	11.52	5.83	1.50	0.63	6.19	8.09	10.06	2.39	0.17	0.20
CV%	48.78	42.00	14.99	33.27	22.43	23.46	19.35	41.90	26.83	25.56
Min <sup>a</sup>	11.14	4.19	6.55	0.51	17.58	12.51	30.82	1.72	0.51	0.38
Max <sup>a</sup>	59.92	25.84	14.41	2.84	41.48	50.06	71.66	10.83	1.31	1.28

No.: number of samples; SD: standard deviation; CV: coefficient of variation.

<sup>a</sup>Data are expressed as per cent on dry matter.

thanks to the complexes of ash with organic compounds. Our results are comparable with the research of Danieli et al. (2004) and performed better than Fekadu et al. (2010) who obtained  $R^2$  in calibration of 0.99 and 0.86, respectively. RPD values using the independent set of data in prediction are higher than 2.5, suggesting a FT-NIRS potential use for ash content determination.

Lipids, expressed as ether extract, obtain a  $R^2_c$  of 0.989 in calibration confirmed in independent validation ( $R^2_p$ : 0.982) as well, whereas  $R^2$  for CVm performed worse ( $R^2_{CV}$ : 0.924). The high  $R^2$  found are possible thanks to characteristic aliphatic  $-CH$  adsorption. Nevertheless, in grass samples, lipid prediction is uncommon due to low tissue concentration and to the narrow ranges in forage plants. Roberts et al. (2003) reported that the calibration of lipids in different samples of forage always shows lower result, whereas Stuth et al. (2003) suggested mixed results in the forage measures due to low variance linked to small concentration.

Crude fibre and fibrous fractions can be considered in NIRS spectra due to the  $-CH$  and  $-OH$  adsorptions, even if only lignin is a primary constituent of the forages, whereas the other parameters are properties representing various cell wall components. Crude fibre is the fibrous parameter with the lowest coefficient of determination ( $R^2_c$ : 0.979;  $R^2_p$ : 0.960;  $R^2_{CV}$ : 0.939). However, our CAM results are similar and slightly better for CPM with respect to Danieli et al. (2004). The result of CF estimation is probably associated with the primary chemical analysis method (Weende) that allows only an approximate estimation and that negatively influences the next prediction of NIRS. This problem has been proposed by many authors who reported that one of NIRS application limit is the precision and repeatability inadequacy of the conventional reference methods (Roberts et al. 2003). However, CF is less commonly proposed in forages than in other animal feeds (Roberts et al. 2003) and it is replaced by acid detergent fibre and neutral detergent fibre (Stuth et al. 2003).

Acid detergent fibre shows a slightly better coefficient of determination and root-mean-square error both for calibration ( $R^2$ : 0.991, RMSE: 1.120) and validation models ( $R^2_p$ : 0.960, RMSEP: 1.960;  $R^2_{CV}$ : 0.948, RMSECV: 1.140) than CF. Our ADF coefficients are better than Danieli et al. (2004), Andrés et al. (2005) and Fekadu et al. (2010), which in calibration reported  $R^2$  of 0.97, 0.95 and 0.75 and error of 1.10, 8.46 and 2.95, respectively. For the other parameters, in cross-validation, the RMSECV is higher when compared to the use of independent set of validation but always lower

to the values found in other studies (Danieli et al. 2004; Andrés et al. 2005; Fekadu et al. 2010).

Neutral detergent fibre, fraction not soluble of the grass cell wall composed of different structural carbohydrates and lignin, shows a coefficient of determination of 0.994 and 0.990 in calibration and with an independent dataset of validation with error of 1.150 and 1.330, respectively. In prediction, Danieli et al. (2004) considering a comparable range but with higher number of samples showed only a slightly lower error (2.21). Despite, Andrés et al. (2005) predicted NDF content more accurately than the CP, connecting these results with the high variability of population. Our study shows a lack of precision in cross-validation with a RMSECV of 2.93, but also Fekadu et al. (2010) with a similar number of samples obtained a higher error of 4.97.

Acid detergent lignin shows a  $R^2$  included between 0.991 and 0.926 and RMSE always lower than 1 performing always better than ADF as related to Garcia-Ciudad et al. (1993). Roberts et al. (2003) reported variable results for lignin and higher errors when digestible lignin procedure is considered. Ours results for ADL are always higher than those reported by Danieli et al. (2004), Andrés et al. (2005) and Fekadu et al. (2010) which attributed the not satisfactory results to the negative influence of chemical methods used as reference.

RPD values for fibrous parameters are always high and larger than the limit of 2.5 suggested by Williams and Sobering (1993), showing that it is possible an accurate estimation of these parameters by FT-NIRS.

Milk and meat forage units are poorly estimated probably due to the absence of direct absorption in the near-infrared region. Indeed, these parameters are not directly represented in the spectral region and consequently the calibration is the result of a mathematical derivation. According to Alomar et al. (2009), for an accurate and robust model of estimation, the spectrum must collect direct signals from the chemical bonds that are representative of the parameter. In addition, RPD shows modest values for the forage units, with values between 1.5 and 2.0 that indicate only the potentiality of the model to distinguish between high and low values (Goldshleger et al. 2013).

## Conclusions

Estimation of chemical components and nutritive values of natural pastures of Tuscany, performed using FT-NIRS and PLS models, showed good estimative capacity for all parameters of the chemical composition in terms of coefficient of determination, root-mean-square

error and RPD. The results of CPM model are in line with those related by other researches, whereas in CVM model, results are less comfortable probably due to high variability of samples.

The best estimates were obtained for protein and dry matter followed by lignin and lipid while the lower estimation capacity is shown for the ash components. Thus, if the parameters are not directly represented in the spectral region, the capacity of FT-NIRS seems reduced. A good NIRS estimation must collect direct signals from the chemical bonds of the spectrum that is representative of the parameter.

The application of FT-NIRS has shown results able to estimate the chemical composition of the pastures with accuracy and precision, even with a reduced number of samples. However, for some parameters, milk and meat forage units may be useful for further evaluation.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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